Chapter (non-refereed)

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A critical evaluation of the cotton strip assay

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1 *Summary*
The structure and decomposition of cellulose are discussed, and the traditional definition of cellulose decomposition as the release of glucose units is upheld. The cotton strip assay is described, and it is concluded that changes in tensile strength of cotton strips cannot be related directly to specific biochemical processes which would be of interest to soil biologists.

The broader question of cellulose decomposition in soils is discussed, and the disruption to soil physiological processes caused by adding pure cellulose, in whatever form, is noted.

Published applications of the cotton strip assay are cited, and appear to be associated with poor conceptual models of soil physiological systems. It is concluded that the rate of breakdown of pure cellulose added to soil cannot provide an index of litter decomposition rate, release of litter nutrients, or 'general biological activity'.

2 *Introduction*
The cotton strip assay (Latter & Howson 1977; French & Howson 1982) was put into practical use before it was properly evaluated and before the relationships, if any, between tensile strength change of cotton cloth and soil processes relevant to soil research programmes were examined. Even now, some 18 years later, this situation still holds, and the significance of tensile strength changes in terms of soil processes is a matter for conjecture. The purpose of this paper is to discuss (i) the structure and biochemical decomposition of cellulose, (ii) cotton strip tensile strength changes in relation to enzymic degradation, and (iii) published applications of the cotton strip assay, and to examine the wider aspects of the measurement and importance of cellulose decomposition in soils.

Perhaps the clearest expression of the reasons for using the assay is given by Walton and Allsopp (1977): (i) cellulose is a major constituent of plant remains; (ii) the decomposition of dead plant remains is a major biological process, of great interest to many scientists studying soil processes; (iii) cellulose provides an important food source for a wide variety of organisms; and (iv) a method is needed to compare rates of breakdown of cellulose in different soils. Cotton is a natural substrate, and degradation of any material must begin with bond breaking, which leads to changes in tensile strength. Walton and Allsopp considered that, as long as this technique is used for comparative assessments of biological activity in different soils, it will remain a powerful tool for field research.

3 *The structure and decomposition of cellulose*
Cellulose is a homopolymer, consisting of glucose moieties joined in β-1, 4 linkages, the number of glucose moieties in a molecule being the degree of polymerization (DP). Native cellulose from higher plants has a DP of about 14 000, and this value appears to be remarkably constant. X-ray and infra-red data suggest that the basic structure of cellulose involves the anhydrocellobiose unit ($C_{12}H_{20}O_{10}$) rather than the anhydroglucose unit ($C_6H_{10}O_5$), so that the shortest cellulose-like molecule would be cellotetrose (DP = 4):

$$(C_6H_{11}O_5) \,.\, (C_{12}H_{20}O_{10}) \,.\, (C_6H_{11}O_6)$$

Here, $(C_6H_{11}O_5)$ represents a glucose moiety with a free secondary hydroxyl group on C-4, and $(C_6H_{11}O_6)$ is a glucose molecule with a reducing group. Cellobiose, cellotriose, and cellotetrose are water-soluble, cellopentose is very sparingly soluble, and cellulose molecules with DP greater than 6 are insoluble (Ljungdahl & Eriksson 1985).

Because cellulose is a polymer, we have to think rather carefully about how we define cellulose decomposition. In nature, cellulose is degraded by a range of aerobic and anaerobic fungi and bacteria, many of which occur in extreme conditions of temperature and pH. A list of fungi which have been examined in cellulose decomposition studies is given in Ljungdahl and Eriksson (1985). Many fungi can utilize oligosaccharides and polysaccharides as carbon sources, but, in general, these molecules are too large to be transported directly into the cell, and must first be hydrolyzed to their subunits. Cellulose represents an important potential carbon and energy source for fungi, many of which produce a series of enzymes, collectively called cellulase (Sagar 1988), which facilitate the degradation of cellulose to glucose units.

Cellulose-degrading enzyme systems have been studied in detail in 2 fungi, *Sporotrichum pulverulentum*, the conidial state of the white-rot fungus (*Phanerochaeate chrysosporium*) (Eriksson 1981), and the mould *Trichoderma reesei* (Ryu & Mandels 1980). They have similar hydrolytic enzyme systems with at least 3 components:

i. endo-1, 4-β-glucanases, which split randomly 1, 4-β-glucosidic linkages within the cellulose polymer;
ii. exo-1, 4-β-glucanases, which split off either cellobiose or glucose from the non-reducing end of the cellulose polymer;
iii. 1, 4-β-glucosidases, which hydrolyze cellobiose and water-soluble cellodextrins to glucose.

However, relatively little plant cellulose occurs in the more or less pure form, for much of it is encrusted by lignin, hemicelluloses, and pectins. Some white-rot basidiomycetes selectively remove lignin and hemicellulose without degrading much cellulose, whilst others remove all the cell wall components (Otjen & Blanchette 1985).

Brown-rot fungi are generally basidiomycetes and decompose mainly wood polysaccharides. They produce component (i) but lack (ii) (Highley 1975a, b), and the biochemistry of cellulose degradation by them is not fully understood. It has been found that brown-rot fungi can attack cellulose at some distance from the fungal cell wall, and the initial attack could be by low molecular weight substances that diffuse easily through wood cell walls. Brown-rot fungi degrade cellulose in wood, but they do not seem to degrade pure, isolated cellulose (Nilsson 1974a; Highley 1977). Highley (1978) found that, in culture, the brown-rot fungus (*Poria placenta*) could not digest crystalline cellulose, unless glucose, starch, holocellulose, or mannose was added. Hydrolysis of crystalline cellulose is thought to occur with endo-glucanases attacking randomly over the cellulose polymer, creating end groups for the exo-glucanases to attack from the non-reducing ends of the chains. Degradation of highly ordered cellulose in native cotton fibres depends almost completely on the synergistic action of the 3 enzyme systems listed above (Sagar 1988). None of them acting independently can solubilize cotton to a significant extent (Eriksson & Wood 1984).

Bacterial cellulase systems appear to be more complex than those of fungi, and they cannot be characterized neatly in terms of endo-1, 4-β-glucanases and exo-1, 4-β-glucanases. Many bacteria do not possess β-glucosidase (cellobiase) or, if they possess it, they may still metabolize cellodextrins and cellobiose by phosphorylases (Ljungdahl & Eriksson 1985). In the end, in aerobic conditions, the result is the formation of glucose.

So, how do we define cellulose decomposition? A reduction in the DP, brought about by the action of the endo-glucanases, results in molecules which are still, by definition, cellulose, until the DP falls to a very small value. Cellulose molecules with a DP greater than 4 are very sparingly soluble or insoluble. Many micro-organisms can use glucose, and the reduction of the cellulose molecule to its basic glucose units is the logical definition of cellulose decomposition. This definition has been used by soil researchers for many years, and cellulose decomposition has long been measured by glucose production.

4 *The relationships of cotton strip tensile strength changes to soil processes*

The structure of cotton fibres is complex (Sagar 1988). It seems to be generally agreed that the glucose units are linked together to give a somewhat kinked but rather rigid chain, about 2000 nm long and 0.75 nm wide. However, this structure alone is not sufficient to account for the physical properties of cellulose fibres (Rogers 1961). The strength of cotton fibres depends on many structural features, such as the average chain lengths of the molecules, their homogeneity, and orientation. A close correlation has been found between the strength of a fibre and the degree of polymerization of the component cellulose molecules (Siu 1951). Bundles of chains are thought to be held together by hydrogen bonds and van der Waal's forces, to form microfibrils about 5 nm in diameter. Few, if any, cellulose chains cross from one microfibril to another, and the microfibrils are built up into layers (Selby 1968).

The mode of breakdown of cotton fibres by soil organisms is not fully understood. Work cited in Section 3 suggests that enzymic attack by endo-glucanases would progressively reduce the DP, and the synergistic effect of these, the exo-glucanases, and the glucosidases would lead eventually to glucose. Random severing of the glycosidic linkages of the cellulose polymer would bring about a reduction in both the chain length of the molecules and their homogeneity. In this way, there would be an initial fall in tensile strength, without much loss of glucose units. The relationship between tensile strength and weight loss suggests such an effect (Siu 1951; Selby 1968; Heal *et al.* 1974). There is also evidence that the inaccessibility of cotton fibres to large protein molecules delays enzymic attack and results in the initial breakdown being localized (Sagar 1988). Nilsson (1974b) found that 15 out of 20 fungal species tested could produce cavities in cotton fibres, and 18 species could produce surface erosion. Localized effects of this type will reduce tensile strength with small weight loss.

That such changes do actually occur was shown by Halliwell (1965), who found that enzymic degradation of purified cotton fibres resulted in the release of insoluble, non-filter-passing, very short fibres; insoluble, filter-passing products; and soluble products, mainly glucose.

It has been suggested that cotton strip tensile strength changes might be calibrated in terms of weight loss. The results of Halliwell show that weight loss itself is not readily interpretable; as much of it represents products of partial decomposition. Ghewande (1977) studied loss in weight of filter paper and of cotton in cultures of 5 plant pathogenic fungi. For all the fungi, loss in weight of filter paper was greater than that of cotton, and the ratios of the weight losses differed for the different fungi. Different artificially processed cellulose materials seem to be attacked in different ways. In general, there was no correlation between loss in weight of the cellulose and cellulase production by a fungus (Ghewande & Deshpande 1975).

5 *Application of the cotton strip assay*

5.1 Its use in ecological studies

Rovira (1953) measured changes in tensile strength of cotton tapes buried in soil, but his method has not been much used. The first recorded ecological use of the assay, as described by Latter and Howson (1977), appears to have been by Springett (1971), for assessing 'relative decomposition rates' in respect to maritime pine (*Pinus pinaster*) litter. Latter and Howson (1977) described the method as used in the tundra biome of the International Biological Programme, for assessing cellulose decomposer activity (Baker 1974; Heal *et al.* 1978). Other users have been Wynn-Williams (1979, 1980) to study cellulose decomposition in antarctic soils; Miles (1981) and Miles and Young (1980) to study cellulose decomposition in heathland and moorland soils colonized by birch (*Betula* spp.); and Brown *et al.* (1983), Brown (1988) and Brown and Howson (1988) to assess the 'potential for decomposer activity' in soils under alder (*Alnus glutinosa*), Scots pine (*Pinus sylvestris*), oak (*Quercus* spp.), and Norway spruce (*Picea abies*).

5.2 Difficulties in interpretation of results

Several points which have arisen in various papers indicate that there are difficulties in interpretation, in terms of soil organic matter decomposition processes.

Heal *et al.* (1974) and Latter and Howson (1977) have computed a series of regressions relating change in tensile strength of cotton strips to weight loss of cotton following decomposition. Though the relationships were statistically significant, in some cases highly significant, there were also very significant differences in the regressions for the different sites studied. The authors themselves state that 'a strict comparison between TS and weight losses cannot be expected; weight loss represents an average measure of decomposition over the whole test piece, whereas the tensile strength is a measure of loss at the most decomposed part'.

There appears to be no correlation between the decomposition of cellulose and cellulase activity in soils (Ross *et al.* 1978; Ross & Speir 1979).

Heal *et al.* (1974) used the cotton strip assay, essentially as described in Latter and Howson (1977), for relating decomposition potential to weight losses of litter. They found that (i) with low weight loss of cotton strips, there was a very rapid loss of tensile strength, (ii) in very wet conditions, there was a decline in tensile strength loss of cotton strips, although litters continued to show high weight losses under the same conditions, and (iii) there was an important effect of litter quality in the processes of decomposition.

5.3 Untenable arguments put forward to justify use of the assay

5.3.1 Cellulose is a major constituent of plant detritus

The fact that cellulose is the major constituent of plant remains does not justify the use of cotton strips, or any other pure and, especially, processed cellulose substrate, to simulate the decomposition of cellulose in plant remains. Plant cellulose does not enter the soil in pure form; most plant cellulose is encrusted with lignin and hemicelluloses. Plant material added to soil in natural conditions always contains some nitrogen (N), and materials from various origins have characteristic carbon/nitrogen (C/N) values. Populations of soil organisms develop during the degradation of these materials, and the resulting soil organic matter also has characteristic C/N values. The response of the soil biota to the pure cellulose substrate will necessarily differ from that to plant litters. If anything is needed in such studies, it is a method for studying the decomposition of cellulose, and other constituents, in plant litter.

5.3.2 The decomposition of dead plant detritus is a biological process of considerable importance in maintaining soil fertility

The idea that cellulose decomposition can represent litter decomposition adequately is clearly a gross oversimplification. Minderman (1968) showed that different litter constituents decompose at different rates, and accumulation of residues is determined mainly by the content of intractable constituents. Various authors have preferred to use lignin content as a predictor of litter decomposition rate (Fogel & Cromack 1977; Meentemeyer 1978; Melillo *et al.* 1982). Changes in log biomass were also correlated with lignin content (Charley & Richards 1983). Berg and Staaf (1980) found that, in the early stages of decomposition of Scots pine needle litter, ie up to 30% weight loss, loss in weight was due mainly to soluble substances and degradation of cellulose and hemicellulose and was correlated with the initial levels of N, phosphorus (P), potassium (K) and sulphur (S). Decomposition of lignin was unaffected by initial nutrient levels, and its decomposition started sooner in litters with much lignin than in those with little. In the later stages of litter decomposition, weight loss depended on lignin decomposition.

It is a matter of common observation that litters of different tree species decompose at different rates on the same soil type, and that litter of a given species decomposes at different rates on different soil types. The rate of litter decomposition is not a property solely of the litter, but is the result of a complex interaction between plant species and soil chemistry (eg Coulson *et al.* 1960; Davies *et al.* 1964). Similar effects appear to occur in grasslands, though perhaps they are less pronounced (eg Barratt 1965).

5.3.3 Cellulose provides an important food source for a wide variety of organisms

Cellulose is certainly an important carbon and energy source for micro-organisms, but polysaccharide molecules are too large to be transported into cells, and cellulose must first be broken down to glucose units.

Because of the structure of cotton fibres and the mode of action of cellulases on them, tensile strength change cannot be related to glucose release.

The degradation of any (purified cellulose) material must begin with bond breaking, leading to changes in its tensile strength, but this has more to do with the properties of textiles than with the study of the transformation of plant remains in soils. The importance of cellulose breakdown in soil systems lies in the fact that it provides a source of carbon and energy for a range of soil organisms, but they can only use the glucose formed. Changes in the tensile strength of cotton strips are closely related to the DP, which is reduced by the bond breaking action of the endoglucanases. However, the resulting material will still be cellulose, and needs to be broken down into the constituent glucose units before it can be used by the micro-organisms. As this stage cannot be related to tensile strength changes, the latter do not provide information which is of use to soil biologists.

An accurate method for determining cellulose decomposition in soils, by measuring the release of glucose units, has existed for some years (Benefield 1971) and is discussed by Ross (1974). Broadly, the method involves incubating cellulose powder with a known quantity of soil in a buffer solution containing Penicillin G as a bacteriostat. After incubation, the glucose formed is dehydrogenated by glucose oxidase, and the hydrogen peroxide so formed is catalyzed by peroxidase to oxidize o-tolidine dihydrochloride. The latter reaction is determined colorimetrically. Penicillin G is thought not to suppress fungal activity, so the method measures the activity of cellulase enzymes present initially in the soil sample, plus any which were synthesized during the incubation. There are important physiological differences between the cotton strip assay and the normal methods for determining cellulase activity. Although cellulase enzymes are inducible, cellulase methods such as that of Benefield (1971) use a short incubation time, which is unlikely to allow major changes in the quantity of cellulase to occur. Therefore, we may expect such a method to reveal the current propensity of the soil to decompose cellulose.

Figure 1 shows the mean cellulase activities (oven dried (OD) basis), determined by the method of Benefield (1971), of 48 soils (0–5 cm) in and around the English Lake District, sampled at 4-weekly intervals for 56 weeks. Each point in the Figure is the mean of 14 samples (Howard & Howard 1985). Figure 2 gives the same results for loss-on-ignition (LOI) basis. These data make the results for the different soils more comparable as they vary considerably in organic matter content. At any given pH value above pH 4, there is clearly a considerable range of cellulase activity; below pH 4, the range is smaller. However, these results are for soils at 0–5 cm depth, and we have to consider the meaning of cellulase activity at that depth in soils of different pH. Above pH 5, we are dealing with mull soils. Litter deposited on the soil surface does not stay there for long, but is pulled into the soil by the large earthworms (Lumbricidae). Thus, most of the litter cellulose decomposes in the soil. At the same time, fine roots are being formed and decomposed there also. In such soils, we would expect a

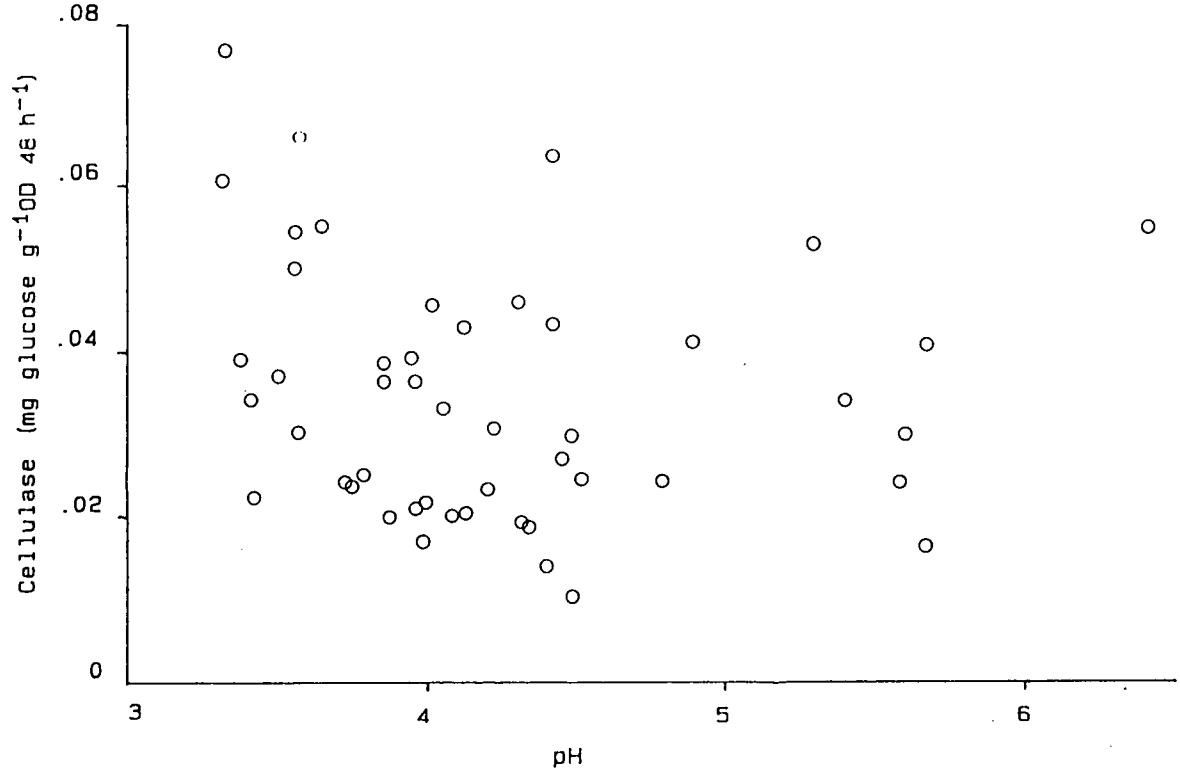

Figure 1. Plot of cellulase activity (OD basis) on pH. Means of 14 4-weekly samples of soils in 48 woods in and around the English Lake District. r = –0.137 NS, r^2 = 0.019

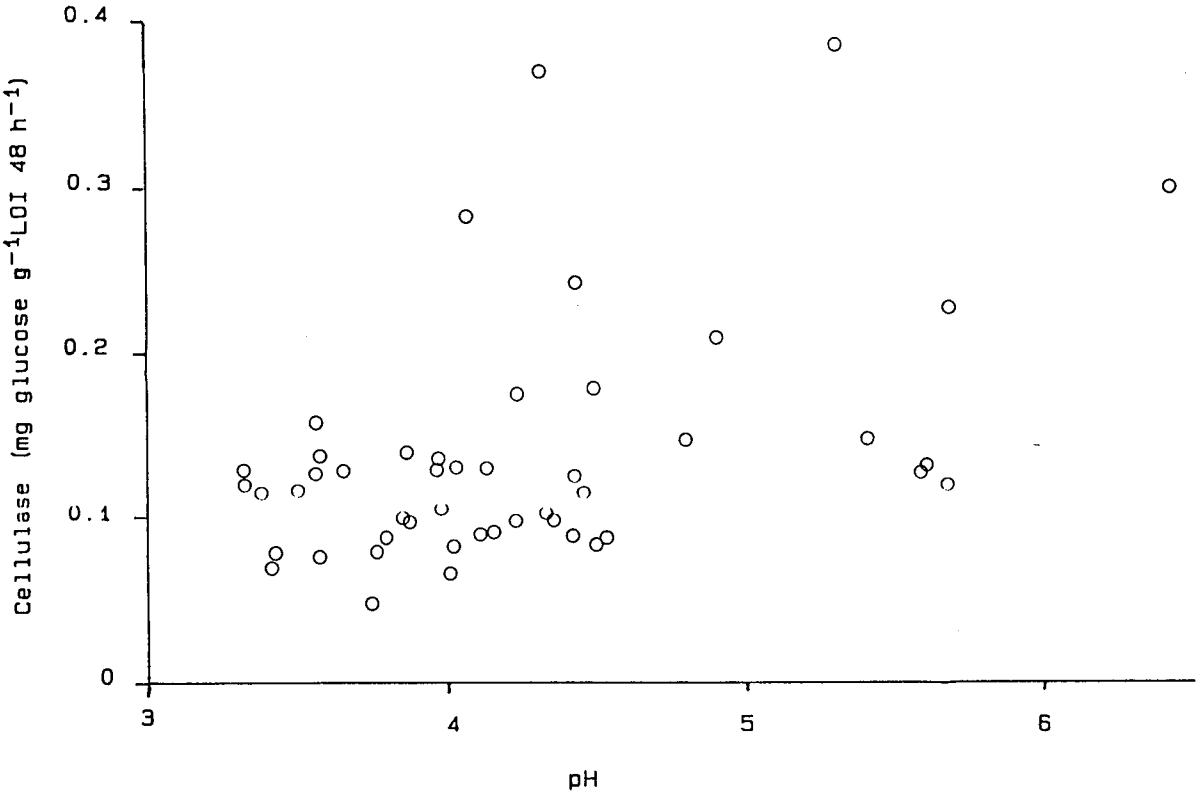

Figure 2. Plot of cellulase activity (LOI basis) on pH. 48 soils as in Figure 1. r = 0.457 P<0.01, r^2 = 0.209

generally high level of cellulase activity. By contrast, below pH 4, mixing of litter with mineral soil does not occur, and the litter cellulose decomposes in the superficial organic layers. Thus, cellulase activity in the surface mineral soil must be largely associated with root decomposition.

This is an important difference in cellulose decomposition in these extreme soil types, and must be recognized when interpreting the results of cellulase activity determinations. The reasons for the wide range in cellulase activities of the soils with pH greater than 5 (Figure 2) may, therefore, lie in differences in quantities of leaf and root material added to the soil. The differences in soils with pH less than 4 may be due to differences in root turnover.

By contrast with cellulase activity measurements, cotton strips are inserted into the soil in the field and left in position for weeks, which allows time for major changes in populations of organisms, and their biochemistry. The capacity of a soil to decompose cellulose is not a fixed property, like the capacity of a cow to eat hay. If we add more cellulose to a soil, the soil organism population will grow to deal with it, but this takes time. As cellulose contains no N, we would expect the addition of pure cellulose to put a severe N stress on the soil. Mull soils with high levels of N mineralization could cope with such stress better than mor soils with low levels of N mineralization. However, the real situation is not so simple, as most cellulolytic fungi show greatly reduced production of cellulases as substrate C/N increases. The white-rot fungi are exceptional in this regard, being able to produce cellulases at a C/N of 2000 (Charley & Richards 1983, p34). It is clear that adding pure cellulose to soils stresses the physiology in ways which are largely unpredictable, making the results difficult, if not impossible, to interpret without a considerable amount of additional information. Cotton strips are likely to be decomposed in soil by a selected microflora, as indicated by Widden *et al.* (1986), and the results obtained by the assay may show little comparison with normal decomposition of plant litter or soil organic matter.

5.4 Its use as an index of 'biological activity'

It has been suggested that cotton strips may be used to provide an index of 'biological activity' of soils, eg when comparing soils under different vegetation types. The idea that the rich diversity of soil physiological activities can be represented adequately by any single measurement can be dismissed by available evidence. Plots of cellulase activity against coefficient of humidity (moisture content g^{-1} LOI), oxygen uptake, and phosphatase activity (both on LOI basis) for the 48 soils in Figures 1 and 2 are shown in Figures 3–5. The largest correlation coefficient is cellulase and pH (r^2 = 0.209).

When the mean values of the soils from the 48 woods were divided into 3 pH groups, <3.8, 3.8–5.0, and >5.0 (Pearsall 1938, 1952), there was no significant difference between the mean values of the groups with regard to humidity. However, for oxygen uptake (LOI basis), the mean of the pH <3.8 group was significantly lower than that of the intermediate group (19.9, 23.8, 23.6 respectively). For cellulase activity,

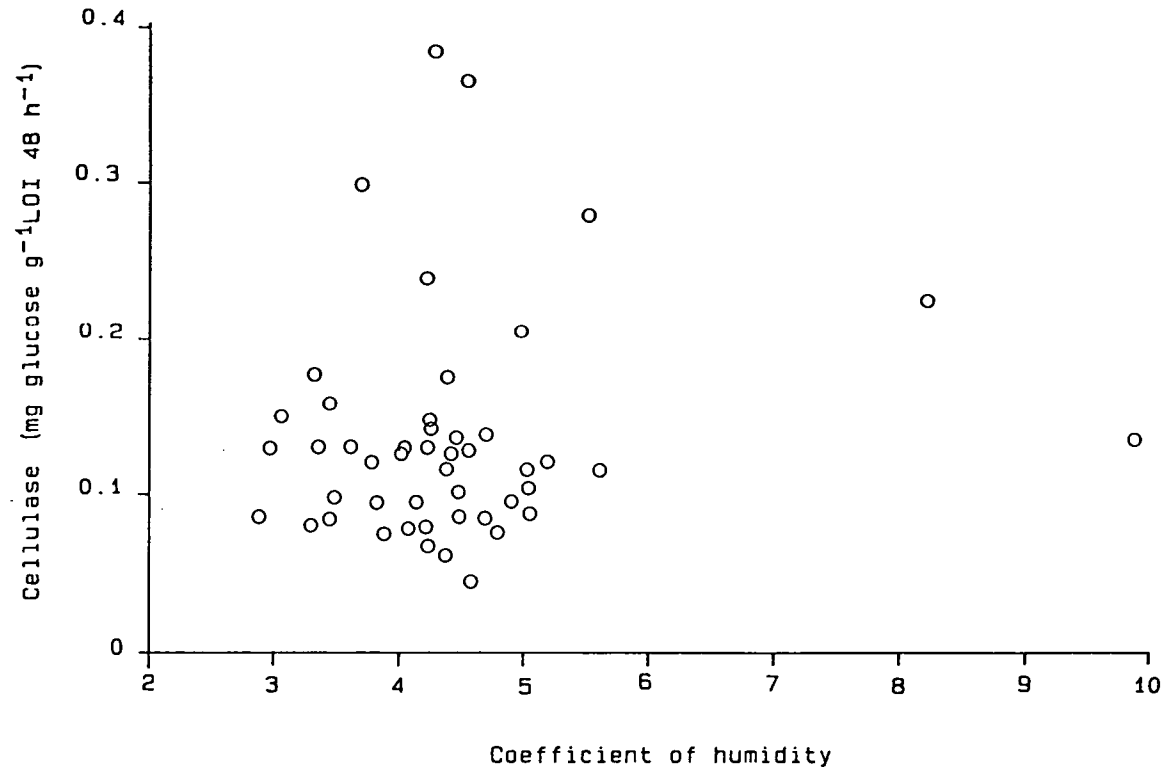

Figure 3. Plot of cellulase activity (LOI basis) on coefficient of humidity (moisture content g^{-1} LOI). 48 soils as in Figure 1. r = 0.122 NS, r^2 = 0.015

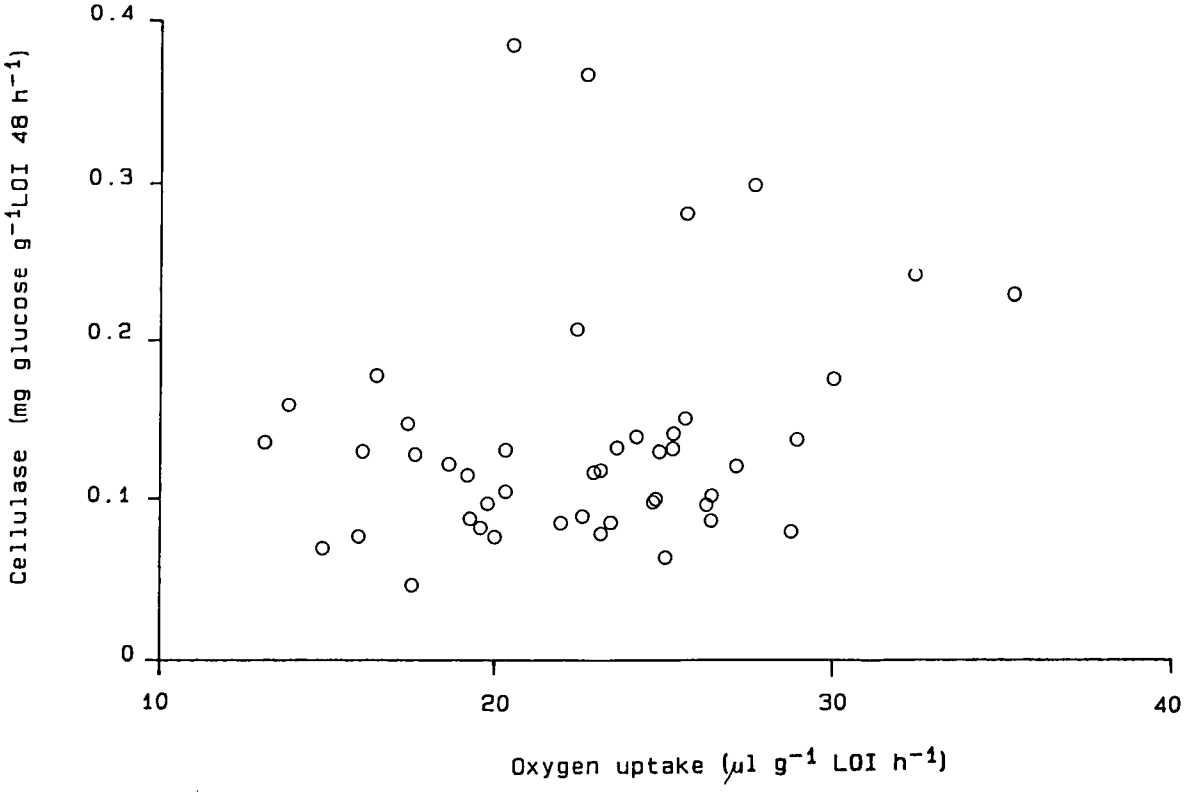

Figure 4. Plot of cellulase activity on oxygen uptake (both on LOI basis). 48 soils as in Figure 1. r = 0.226 NS, r^2 = 0.051

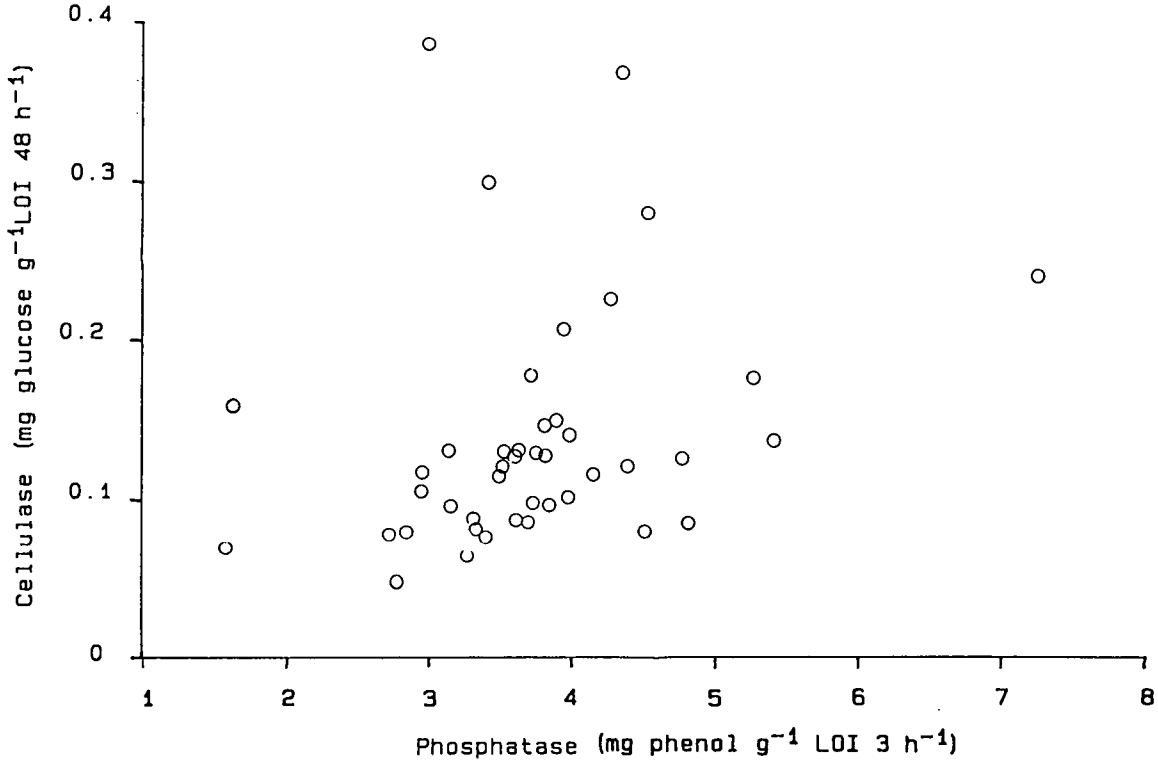

Figure 5. Plot of cellulase activity on phosphatase activity (both on LOI basis). 48 soils as in Figure 1. r = 0.280 NS, r^2 = 0.078

the mean of the more acidic group and the mean of the intermediate group were significantly lower than the mean of the pH >5.0 group (0.106, 0.136, 0.207 respectively). For phosphatase activity, the mean of the more acidic group was significantly lower than that of the intermediate group (3048, 4047, 3757 respectively).

Studying enzymes extracted from coniferous leaf litter, Spalding (1977, 1980) found that cellulase activity was poorly correlated with invertase (r = 0.33, r^2 = 0.11), β-glucosidase (r = 0.37, r^2 = 0.14) and polyphenoloxidase (r = 0.29, r^2 = 0.08). Other workers have found correlations among soil physiological activities to be good in some cases but not in others (Ladd & Butler 1972; Hankin *et al.* 1974; Voets *et al.* 1975; Nannipieri *et al.* 1978, 1979; Frankenberger & Dick 1983). Furthermore, significant correlations between activities observed in one year may not be found in another (Hersman & Temple 1979).

Figure 2 shows that similar cellulase values are possible in the pH range 4–6. Soils at the extremes of this range have greatly different chemical and biological properties. Also, at any given pH, a wide range of cellulase activities is possible. Figures 4 and 5 show that a range of values for oxygen uptake and phosphatase activity is possible for a given cellulase activity, and a range of cellulase activities is possible for given values of oxygen uptake and phosphatase activity. It seems clear that the fact that soils have different cellulolytic activities cannot be used to make deductions about their other physiological properties. Even if the cotton strip assay gave an adequate measure of cellulose breakdown, its use in studies such as those of Brown and Howson (1988) would be pointless.

5.5 Its use in nutrient cycling studies

It has been suggested that the cotton strip assay may be used to indicate differences in nutrient cycling under different soil/vegetation systems, for example in ITE's studies of the Gisburn Forest (Brown 1988) and of grasslands on Plynlimon, central Wales (G Howson pers. comm.). However, no plant nutrients are bound to cellulose, and none would be released by cellulose decomposition. K occurs mainly in vacuolar sap, calcium (Ca) and magnesium (Mg) occur in pectates, and Mg also occurs in chlorophyll and enzyme systems. K and Ca accumulate in stemwood, while P and N occur mostly in the nucleus and cytoplasm.

Brown and Howson (1988) present results from Gisburn Forest which show that changes in tensile strength of cotton strips reflect the growth of trees, either pure or in mixtures. As the tensile strength changes cannot be interpreted directly, a separate study is required on the factors affecting the rate of rotting of the cotton strips, and to investigate if those factors could account for the observed differences in tree growth. It would seem to be simpler to leave out the intermediate step, and to study directly the factors affecting tree growth. A simple explanation for these results would be that the rate of rotting of cotton strips and the growth of trees have nothing in common, except that they both depend on the amount of available soil N (see Carlyle & Malcolm 1986a, b).

6 *Conclusions*

Most published uses of cotton strips are somewhat vague about what the method is intended to show. Phrases such as 'relative decomposition rates' or 'potential for decomposer activity' are common. In general, the assumption seems to be that changes in tensile strength of cotton strips are in some way related to plant litter decomposition.

The use of pure processed cellulose as a surrogate for plant litter is based on highly oversimplified conceptual models of soil physiological systems. Such models stress quantitative aspects, while ignoring important qualitative differences (cf Romell 1935).

Furthermore, plant cellulose is not associated with nutrient elements, and its decomposition is not directly relevant to nutrient cycling. The rate of breakdown of pure cellulose is not strongly related to many other important soil physiological processes, so it cannot be used as an index of biological activity.

In my opinion, the use of an unsuitable method for assessing cellulose decomposition, in association with poor conceptual models, is unlikely to increase our knowledge of the functioning of soil biological systems, or our ability to predict the consequences of natural or artificial perturbation.

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